

Repellent properties of white sagebrush (Artemisia
ludoviciana Nutt. (Asteraceae)) against rice weevil
(Sitophilus oryzae (L.)) and lesser grain borer
(Rhyzopertha dominica (F.))

by

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INTRODUCTION

In the recent past improvements have been made in the production of low cost food and fiber, but very few people are aware of the never-ending battle to achieve this goal. It is a common understanding that better agricultural techniques, new varieties of crops and use of fertilizers and growth regulators have made improvements in crop production. Not only these factors, but also the important factor of pest control has contributed to the success of modern agricultural systems. There are about 10,000 species of insects and related arcnids which at one time or another have caused significant agricultural damage. On a world-wide basis the insect pests annually damage or destroy about 15% of total potential crop production, with a value of more than \$ 35 billion; enough food to feed more than the population of a country like India (Mandava, 1985).

Many pest control techniques and methods have been employed. Of these, use of pesticides has been the most prevalent and efficient method. Unfortunately, recent research has indicated the adverse effects of these chemicals on animals and humans. Although pesticide use is an economical and fast acting method of pest control, its long term use and misuse is dangerous due to its potential health hazards. Researchers have now directed their efforts

to finding new and better ways of pest control that are not only efficient and economical, but also environmentally safe. Naturally occurring pesticides and repellents appear to have a promising role in the development of future commercial pesticides not only for agricultural crop productivity but also for the safety of the environment and public health.

In stored grains and seeds, fumigation with toxic gases has been the major type of control measure against insect pests. Use of these toxic chemicals not only kills the pests, but is also a potential health hazard problem. In addition, use of fumigants requires trained manpower and sophisticated equipment for their application. Another problem is the development of resistance against the toxic chemicals and gases by pest insects.

Biorational control in stored-grain is a natural pest control tactic, which employs the use of repellents, antifeedants or toxicants from natural sources (e.g plant materials) against insect attack. The following study was undertaken for preliminary evaluation of a plant, white sagebrush (Artemisia ludoviciana) as a repellent against two species of stored-grain insects.

REVIEW OF LITERATURE

Use of natural insecticides goes back to the Romans who utilised Fals heleboxe as a rodenticide. The Chinese had employed Thundergod vine (Trypterigeum wildfordie) and Derris spp. as insecticides for hundreds of years. Derris elliptica is a source of the insecticide rotenone which has been used to control Lasioderma serricorne and Callosobruchus maculatus (Chopra, et. al. 1941). The insecticidal properties of Sabadella (from Schoenocaulon spp.) were known in the sixteenth century; tobacco was in use as an insecticide in France prior to 1690; the manufacture of pyrethrins was begun in Europe about 1828; and rotenone was used against nutmeg insects in Singapore before 1848 (Jilani, 1984).

Thousands of higher plant species have been examined for their properties against insects and more than 2000 have shown some activity. Jacobson (1958, 1975) reviewed repellent, antifeedant and insecticidal activity of plants against various insect species. Subsequently Golob and Webley (1980) described the traditional use and results of trials of various plant materials as protectant for stored grains. Many indigenous plants have been used to repel insects. The leaves of neem (Azadirachta indica A. Juss.) and of patchouli (Pogostemon heyneanus Benth.) and roots of

costus (Saussurea lappa C.B. Clarke) were used to protect woolen fabrics from insects (Chopra, et. al. 1941). Neem seed powder mixed with wheat grain at the rate of 1 or 2 percent protected the treated wheat against Sitophilus oryzae, Rhyzopertha dominica and Trogoderma granarium for about 269, 321 and 379 days, respectively (Jotwani and Sircar, 1965). The same rates of application of powdered neem seed protected mung, Bengal gram, cowpeas and peas against Callosobruchus maculatus for about 8, 11, 9 and 9 months, respectively (Jotwani and Sircar, 1967).

Ketkar (1976) used powder of neem seed at the rate of 1 and 2 percent w/w of paddy. He found that oviposition rate of Rhyzopertha dominica and Sitotroga cerealella was reduced and powder mixed at the rate of 0.5, 0.75 and 1.0 percent reduced the population of Sitophilus oryzae, Rhyzopertha dominica and Sitotroga cerealella, respectively. Golob and Webley (1980) reported at least forty different plant species used in the form of powder as protectants from different stored-grain insect pests. Section 1 of this review described the traditional use and trials conducted by different authors with their particular references. Powders of three plants, commonly found in Pakistan, viz. rhizomes of Curcuma longa L. (turmeric), leaves of Azadirachta indica A. Juss. (neem), and Trigonella foenumgraecum L. (fenugreek)

greek) were evaluated for their repellency against adults of Tribolium castaneum Hbst., Sitophilus granarius and Rhyzopertha dominica. Powder of rhizomes of Curcuma longa proved to be the best repellent among the three plant materials tested (Jilani and Su, 1983). The pulverized powder of dill seed (Anethum graveolens) was mixed with undamaged soft winter wheat at the rate of 2, 1 and 0.5 percent by weight. Repellency values obtained by using the food preference wheel method for three doses were each significantly different from untreated samples (Su, 1985). Crushed bay leaves (Laurus nobilis L.) tested as repellent against adults of Tribolium castaneum when added to wheat flour at the rate of 0.22% by weight, gave good repellency (Saim and Meloan, 1986).

Toxicity of many plant powders have been studied by different investigators. Studies on Acorus calamus provided good kills of Sitotroga cerealella and Corcyra cephalonica by treating paddy with rhizome powder tied in a piece of cloth and placed in the center of the bin (Trehan and Pingale, 1947). Abraham et.al., (1972) compared admixture of 1 percent rhizome pieces of Acorus calamus, dried leaves of neem and Vitex negundo. After three months storage, significant difference in percent of paddy grain damaged by Sitotroga cerealella was observed between treated and

untreated lots. Ground black pepper (Piper nigrum L.) applied to soft red winter wheat at the rates of 625, 1250, 2500 and 5000 ppm gave good control of adult rice weevils. Very few F_1 progeny (compared with control) were obtained by five weeks after first emergence (Su, 1977). The same material applied to soft red winter wheat at 1000 ppm provided good protection against S. oryzae up to 6 months. Lower dosages of 500 and 250 ppm did not provide good protection (Su, 1983). Neem leaf/seed powder when applied to shelled maize resulted in reduced F_1 progeny of S. oryzae, Cryptolestes ferrugineus Steph. and R. dominica, but not S. zeamais Mostch. or Tribolium castaneum. Neem seed and leaf powder effeciently reduced adult emergence of Sitotroga cerealella and Ephestia cautella Walk. Neem seed effectively protected maize for three months against S. zeamais and R. dominica but not against S. oryzae (Pereira and Wohlgemuth, 1982).

Jilani and Haq (1984) tested powders of five plant species: rhizomes of Acorus calamus L. and Allium sativum; seeds of Azadirachta indica A. Juss. and Carum copticum; and leaves of Xanthoxylum armatum D.C. as grain protectants against R. dominica, S. oryzae and Sitotroga cerealella. They found that rhizomes of Acorus calamus completely checked the development of insects in wheat grain, while powders of Azadirachta indica and Allium sativum also gave

good results. Ground dried peels of orange (Citrus sinensis L.) and grapefruit (Citrus paradisi Moef.) were tested for toxicity to Dermestes maculatus Deg. and Callosobruchus maculatus F. when applied to cowpea grains. LD₅₀ values obtained were 4.0 gm (peel)/ 100 gm (cowpea) of orange and 5.62 gm (peel)/ 100 gm (cowpea) of grapefruit for Callosobruchus maculatus. LD₅₀ values for D. maculatus were much higher at 14.13 gm (orange peel)/ 100 gm (fish chips) and 14.29 gm (grapefruit peel)/ 100 gm (fish chips). Orange peel at high dosages also depressed progeny development of D. maculatus (Don-Pedro, 1985).

The genus Artemisia (Sagebrush plant) is a large group of small herbs comprising some 400 species distributed in the northern hemisphere (Greger, 1982). In the Western United States Artemisia grows abundantly, whereas in the southern hemisphere (South America and South Africa) there is scattered distribution.

Artemisia ludoviciana (Fig.I) is a perennial member of the Asteraceae family commonly found throughout the Great Plains. It grows in dry soil up to 3 feet in height from Missouri to Texas, Wyoming, Colorado and Arizona. Stem of the plant is woolly, branched above, leaves are linear to obovate which are white woolly beneath. At length leaves are dark green and glabrous, at bases narrowly cuneate, lobed or



Figure 1: White sagebrush (*Artemisia ludoviciana* Nutt. (Asteraceae)). (After Britton and Brown, 1913)

toothed. Upper leaves are often linear and entire; flower heads are numerous, spicate-paniculate, 1-1.5 inch broad. Receptacle is naked and central flowers are fertile. Mature plants are found from August to November (Britton and Brown, 1913).

Greeks and Romans used Artemisia spp. as an anthelmintic and stomachic in ancient times. Persian and Arab physicians also employed Artemisia spp. for the same purpose (Thakur and Singh, 1979). The Yokio Indians of Mendocino county used an indigenous species of this genus for tea of boiled leaves to cure bronchitis. Another species Artemisia frigida was used as a source of camphor. The Kiowa Indians, a plains tribe, chewed leaves of Artemisia mexicana for sore throat, while leaves of black sage were chewed by the Tewa Indians to expel gas from the intestinal tract (Weiner, 1980). One species Artemisia absinthium was used by Italian farmers to protect grains in store houses from Sitotroga cerealella, Calandra spp. and Tinea granella (Ciaravellini D. 1948). Artemisia vulgaris proved to be a promising insecticide when the entire plant was used against various insect species (Petrishcheva, 1945). Insecticidal effects of plant extracts from Artemisia spp. against some delphacid rice pests have also been evaluated (Jabbar Khan, 1984).

About thirty species of Artemisia have been reported to grow naturally in Pakistan (Stewart, 1972). These herbs are of economic importance to Pakistan. Some have medicinal value and yield essential oils, while a few are useful as fodder. Artemisia maritima L. is found in Kurram valley, Gilgit agency, Kaghan valley, Chitral, DirSwat, Khyber agency, Indus, Kohistan and large barren areas of Baluchistan. Santonin, an anthelmintic drug is extracted by Kurram Chemical Company, Rawalpindi, one of the largest manufacturers of this drug in the world (Hasan, 1984). At least ten different species of Artemisia have been studied in Pakistan for their chemical constituents but very little work has been done on the activity of these compounds against insects.

Artemisia ludoviciana has been studied for its anti-feedant activity against some species of grasshoppers. Development and survival of Hypochlora alba were normal on plants with sparse or medium pubescence, but reduced and terminated earlier on those with dense pubescence. This was because younger instars ingested less pubescence in proportion to leaf tissue than did older instars and adults. Field collected Melanoplus bivittatus and Melanoplus sanguinipes attained only 3rd instar on normal leaves (Knutson, 1982). When excised leaves of this plant were fed to two species of grasshoppers, with or without nonglandular

trichomes, survivalship was highest for Hypochlora alba on pubescent leaves and was lowest for Melanoplus sanguinipes. It was found that nonglandular trichomes of Artemisia ludoviciana had less effect on feeding behavior of H. alba than on M. sanguinipes (Smith and Grodowitz, 1983).

Preliminary studies reported here were to evaluate white Sagebrush plant powder as a repellent to two different species of stored-grain insects. Results could lead to the detailed investigation of this plant species as a source of a stored-grain protectant.

MATERIALS AND METHODS

(A) Collection and Preparation of Plant Materials

Artemisia ludoviciana Nutt (Asteraceae) commonly known as white sagebrush was collected from two different sites at Manhattan, Kansas on June 24, 1986: the KSU Animal Science and Industry farm, located west of Manhattan and from the Carnahan Creek area, to the north east of Manhattan. At the time of collection, plants were of variable sizes and at different stages of growth. The plants were actually taken out of the soil with their roots intact. Collected plants were separated on the basis of their size/growth stage into two categories, viz. less than 15 cm in height and more than 15 cm in height. Small plants were used for whole plant testing in which roots, shoots and leaves were intact, but flowers were absent. The larger plants were used only for testing of leaves. Flowers of the plants were collected on August 24, 1986, from the same two sites.

The materials were dried at $25 \pm 1^{\circ}\text{C}$ ($75-77^{\circ}\text{F}$) for one month. The whole plant (small plants), leaves (of larger plants) and flowers (from mature plants) were ground on a Wiley mill by using a screen of 1 mm pore size to get a uniform powder. These powders were sealed in plastic bags and kept in a freezer (-18°C) for later use. The powders of plants collected from two different locations were mixed

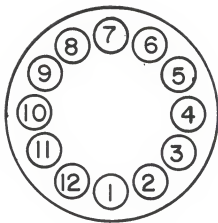
in equal amounts to provide a homogeneous sample before testing.

(B) Rearing of Insects

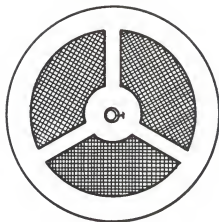
Two stored grain insect species, viz. The rice weevil (Sitophilus oryzae (L.) (Kansas strain)) and lesser grain borer (Rhyzopertha dominica (F.)) were reared in the laboratory at $27 \pm 1^{\circ}\text{C}$ ($80-82^{\circ}\text{F}$) and $60 \pm 5\%$ relative humidity. Hard red winter wheat with moisture content adjusted to 12-13 % (w.b) was used as culture medium for both of the insect species. These insects were reared in quart glass jars having brass screen in the lid and a filter paper inserted inside the lid below the brass screen to avoid any kind of insect contamination in the culture. Two jars of each insect culture were prepared weekly to provide adult insects of known, uniform age.

(C) Preparation of Test Arena

Three choice chambers (Figure II) were constructed by modifying the one used by Laudani and Swank (1954) and Jilani and Su (1983). An aluminum grain grading pan having a diameter of 33.02 cm (13 inches) was used as a circular platform. Twelve holes of 4.4 cm diameter each were made towards the outer edge of this platform equidistant from the center. Each such hole held a cup made of aluminum (44 x



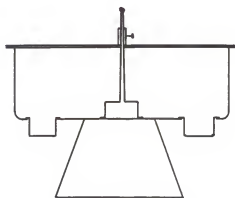
(a)



(b)



(c)



(d)

Figure II: Apparatus used for repellency bioassay.
 a) Platform b) Lid c) Plastic plate
 d) Cross section of whole chamber.

17.5 mm, Fisher Scientific) fitted into the hole with its collar in such a way that it was not possible for insects to escape. The insects were prevented from climbing up the walls of the chamber by putting a thin layer of ground glass (stopcock) grease all around the top of the inside wall, about half an inch wide. A plywood lid was prepared having a 2.54 cm (1 inch) diameter hole in the center through which a plastic tube was placed to introduce the insects. The lid was also provided with a maximum coverage of 100 mesh brass screen. An inverted plastic can, 1.9 liters (0.5 gallon) capacity, was used as the base of the chamber. A transparent plastic plate (6 mm thickness) was prepared having a diameter equal to the inner diameter of the platform. This plate (Fig. IIc) had a cut out section equal to the diameter of one cup.

(D) Entomological Studies

(1) Repellency studies using plant powders

Three plant powder fractions, viz. whole plant, leaves and flowers, were admixed uniformly with hard red winter wheat (moisture content approximately 12.8-13% (w.b)) at six dose levels: 2, 1, 0.5, 0.25, 0.125 and 0.0625 % w/w. Mixing was done in a glass jar (one pint capacity) by stirring with a glass rod and then by placing a lid on the jar and shaking the jar to get a uniform mixture. In each

experiment there were six treated and six untreated samples of grain placed in alternate cups. Each cup contained about 22 gms of wheat sample. In each test, 120 one to two weeks old adult insects were introduced into the choice chamber and allowed to enter cups of their choice for a period of 24 hours. The chambers were placed in a dark room maintained at $27 \pm 1^{\circ}\text{C}$ ($80-82^{\circ}\text{F}$) and $60 \pm 5\%$ relative humidity. After 24 hours, the lid was removed from the choice chamber under a red darkroom light. The flat plastic plate was placed over the choice cups so that the insects were prevented from moving out of the cups until each individual sample was removed. Only one cup was exposed when the plate was in place. The flat plastic plate was rotated to expose each cup just before it was removed from the choice chamber and its contents placed in a petri dish. Insects in each wheat sample were counted outside the darkroom in the laboratory.

Black, 4 mil (0.1 mm) polyethylene sheet was used to provide a light barrier to avoid light affecting choice samples when the door of the dark room was opened. Each test was repeated three times during three consecutive days using newly treated grains and new insects. Different aluminum cups were used for different insect species and were cleaned with cotton between replicates of the experiment. The interior of the choice chamber was cleaned thoroughly with cotton dipped in acetone.

Repellency values were calculated using the formula of Leonard and Ehrman (1976):

$$\text{Percent repellency} = \frac{N_c - N_t}{N_T}$$

where

N_c = Number of insects found in untreated cups.

N_t = Number of insects found in treated cups.

N_T = Total Number of insects found in treated and untreated cups

(2) Incubation Studies

Wheat samples from the 2, 1 and 0.5 % dose levels for leaves and flowers were separated from insects and were incubated in 48 x 48 x 20 mm plastic boxes with brass screened lids. Samples were stored at $27 \pm 1^\circ\text{C}$ ($80-82^\circ\text{F}$) and $60 \pm 5\%$ relative humidity for 60 days. Numbers of adult insects emerged from these samples were counted to determine the effect of treated and untreated samples on possible population increase in treated samples.

(3) Persistence Studies

The residual effect of the flower powder fraction was determined using three dose rates viz. 1, 0.25 and 0.125 %

w/w selected after comparing the data obtained in repellency tests. Flower powder was tested for its persistent effect because it gave significantly better repellency values than the other parts of the plant.

Hard red winter wheat was treated with the indicated doses of flower powder in 2 kg lots. Wheat was admixed with powder of flowers first by stirring with a glass rod and then tumbling about forty times upside down and side ways manually in 3.8 liters (1 gallon) capacity glass jars. Each dose was replicated three times and stored at $27 \pm 1^{\circ}\text{C}$ ($80-82^{\circ}\text{F}$) and $60 \pm 5\%$ relative humidity. Untreated control samples of wheat were stored under the same conditions in the same capacity jars.

Repellency values of samples were determined initially on the day of treatment and at monthly storage intervals for four months. After each month, lots of wheat were thoroughly mixed and samples drawn from each jar using a coffee spoon that contained about 20 grams of wheat sample. Treated and untreated samples were tested for their repellency using the choice chamber method as previously described.

(E) Statistical analysis of data

Data was analyzed using PROC ANOVA and PROC GLM on SAS Programme (SAS Institute Inc., Cary, N.C 27511-8000).

RESULTS AND DISCUSSION

(1) Repellency in free choice tests

Mean percent repellency of different plant parts at six dose levels against rice weevil (RW) and lesser grain borer (LGB) are plotted in Figures III and IV, respectively. Data for both insect species are given in Appendices I and II. Repellency values increased as the dose increased for both insect species and for all plant parts. There was a relatively abrupt increase from the lowest dose of 0.0625% to 0.125% for RW (Fig.III). This was indicative of a break point among the doses. Flowers were more repellent at low dose levels but whole plant powder was as effective at higher doses against RW. Maximum repellency values (near 100%) were obtained at the highest dose of 2% with all plant parts.

The response pattern for LGB was somewhat different (Figure IV). There was a wider range of differences among the plant parts and also dose levels. Here, there was no particular break point at any dose level. With LGB, powder of flowers gave the highest repellency values except at the lowest dose (0.0625%), where it was less than whole plant powder repellency. The lowest repellency value for LGB was 24.24% as compared to 62.01% for RW at 0.0625%. Maximum repellency obtained for LGB was

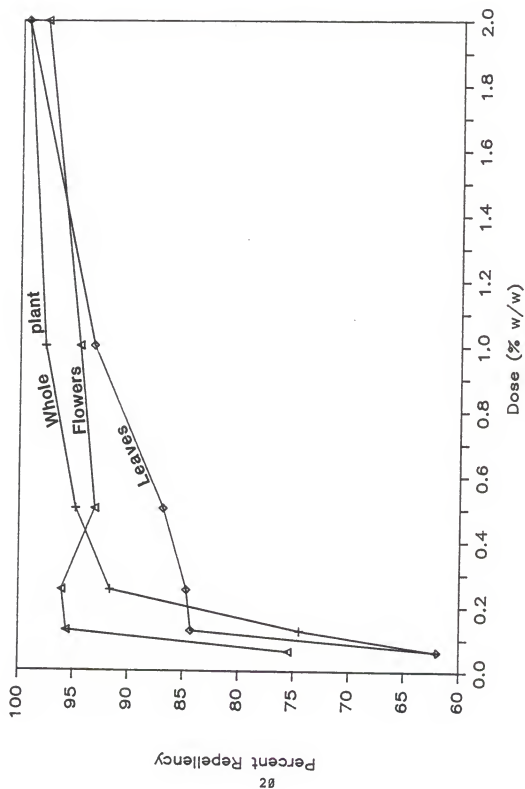


Figure III: Mean percent repellency of different parts of white sagebrush at six dose levels against RW

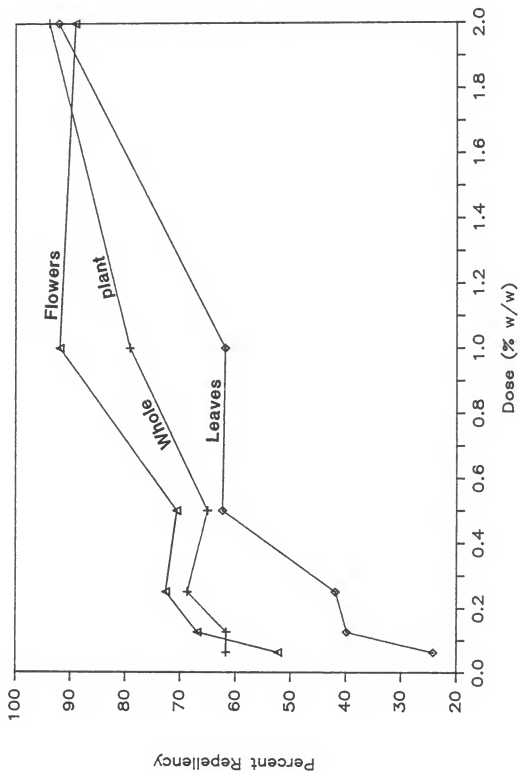


Figure IV: Mean percent repellency of different parts of white sagebrush at six dose levels against LGB

93.86% at the 2% dose level compared to 99.44% for RW. LGB was less responsive to the plant powders used than RW. Similar results were obtained by Sighamony et. al. (1986), where oils of certain indigenous plants were more effective in killing RW than LGB. Qadri (1973) showed that rice weevils were more powerfully repelled than flour beetles when seed powder extracts of some indigenous plants were used in a free choice test.

A statistical comparison of pooled mean percent repellency values at six dose levels for each insect species obtained by analysis of variance is given in Table 1. For RW ($F = 20.93$; $d.f = 5$; $p > 0.0001$), doses of .2 and 1% were not significantly different from each other, but they were different from lower doses of 0.5, 0.25 and 0.125%, respectively. The lowest dose of 0.0625% was significantly different from all the higher doses. Dose levels can be divided into three groups according to their significance: 1) Higher doses of 2 and 1% with significantly higher mean percent repellency values. 2) Middle doses of 0.5, 0.25 and 0.125% with medium repellency values and 3) lowest dose of 0.0625% with lowest mean percent repellency. Middle doses may be viewed as promising sources of repellency against RW.

Pooled mean percent repellency values for LGB were also statistically compared ($F = 16.88$; $d.f = 5$; $P > 0.0001$)

Table 1: Statistical Comparison of Mean Percent Repellency of white sagebrush at different dose levels against RW and LGB

Dose (% w/w)	Mean Percent Repellency* against	
	Rice Weevil	Lesser Grain Borer
2.0	98.87 a	91.70 a
1.0	95.17 ab	77.70 b
0.5	91.62 bc	66.02 c
0.25	90.82 bc	61.10 c
0.125	84.80 c	56.13 cd
0.0625	66.53 d	46.07 d

* Each value is a mean of 9 observations.

Means within columns having same letter are not significantly different at $\alpha = 0.05$

(Table 1). For LGB middle doses of 0.5, 0.25 and 0.125% were not statistically different among themselves, whereas doses of 1.0 and 2.0% gave significantly greater repellency, respectively. An important point to note is that the highest dose provides only that level of repellency achieved by middle doses against RW. Pooled mean percent repellency values at all dose levels were considerably higher for RW than LGB.

Mean repellency values obtained in comparison of three different plant parts against RW and LGB are given in Table 2. Powder of flowers of white sagebrush was statistically most repellent of the three parts against RW. For RW ($F = 4.24$; $d.f = 2$; $P > 0.0223$) flower powder was significantly more repellent than leaf and whole plant powders, which were not significantly different from each other. The situation was somewhat different for LGB ($F = 15.71$; $d.f = 2$; $P > 0.0001$), where flower powder and whole plant powder were both significantly more repellent than leaf powder.

It is important to indicate here that repellency data were analysed in original form and also transformed. Transformation used was $\arcsin \sqrt{\text{repellency}}$. No difference in significance of models or comparisons at $P = 0.05$ was observed between transformed and original data. Significance values for original data are used for discussion here.

Table 2: Statistical comparison of mean percent repellency of different parts of white sagebrush against RW and LGB

Part	Mean Percent Repellency* against	
	Rice Weevil	Lesser Grain Borer
Flowers	92.09 a	73.92 a
Whole plant	86.71 b	71.70 a
Leaves	85.71 b	53.74 b

* Each Value is a mean of 18 observations.

Means within columns having same letter are not significantly different at $\alpha = 0.05$

Another important point is that dose X part interaction was not significant for either insect species (RW; $F = 1.56$, $d.f = 10$, $P > 0.1592$ and LGB; $F = 1.63$, $d.f = 10$, $P > 0.1384$). This tends to confirm that percent repellency was dose dependant with no cross over among the dose levels and various plant parts.

One of the important factors in some behavioral-type studies is the amount of nonresponsive action in the bioassay used. In this research, the number of insects found outside the cups after 24 hours of choice test were considered as "nonrespondents". Data were analyzed to see whether nonrespondents had an effect on percent repellency values. It was obvious that mean numbers of LGB outside cups was very small (out of 120 insects released) and more or less the same for all of treatments (Table 3). No significant effect on percent repellency was obtained when this data was analysed ($F = 0.75$; $d.f = 17$; $P > 0.7332$).

Greater numbers of RW were observed outside the cups (Table 4). There were significant difference between doses ($F = 4.0$; $d.f = 5$; $P > 0.0055$) but not between plant parts ($F = 1.22$; $d.f = 2$; $P > 0.3059$) or dose X parts ($F = 1.62$; $d.f = 10$; $P > 0.1396$). The lowest dose (0.0625%) had the greatest number of insects outside, followed by 0.5, 0.125, 1.0, 0.25 and 2.0% doses (Table 5). A general trend of

Table 3: Mean number of nonrespondent adult LGB after 24 hour repellency test in a free choice chamber

Dose (% w/w)	Mean No. of insects* outside cups using		
	Whole plant	Leaves	Flowers
2.0	0.67	1.67	4.00
1.0	5.00	3.00	3.67
0.5	2.00	3.00	2.00
0.25	3.00	1.33	2.00
0.125	3.67	3.00	6.67
0.0625	2.00	6.33	6.33

* Each value is a mean of three replicates

Table 4: Mean number of nonrespondent adult RW after 24 hour repellency test in a free choice chamber

Dose (% w/w)	Mean No. of insects* outside cups using		
	Whole plant	Leaves	Flowers
2.0	2.00	2.67	2.67
1.0	4.33	1.66	7.33
0.5	6.00	11.67	13.33
0.25	6.67	2.00	1.00
0.125	20.00	1.00	9.00
0.0625	15.67	15.00	8.67

* Each value is a mean of three replicates

Table 5: Statistical comparison of mean numbers of nonrespondent adult RW after 24 hour repellency test at different dose levels

Dose (% w/w)	Mean No. of insects*
0.0625	13.11 a
0.5	10.33 ab
0.125	10.00 ab
1.0	4.44 bc
0.25	3.22 c
2.0	2.44 c

* Each value is a mean of 9 observations.

Means with the same letter are not significantly different at $\alpha = 0.05$

increasing numbers of insects outside cups was observed as the dose rate decreased (Fig. V). The mean value for 0.25% was very low.

One possible explanation for the difference in LGB and RW nonresponse could be related to the normal activity of the insects. RW are generally considered more active than LGB in nature, and it is possible that this difference in behavior is responsible for the observed difference in nonresponse. It is also known that males of LGB and RW produce aggregation pheromones attracting both sexes (Khorramshahi and Burkholder, 1981; Phillips and Burkholder, 1981). Differences in response due to the production/nonproduction of pheromones by the two insect species is probably not the case here.

(2) Emergence of F₁ Progeny

F₁ progeny emergence of RW and LGB from samples exposed in free choice chambers to insects for 24 hours was determined. In the free choice repellency tests, numbers of insects were counted in different treatments after 24 hours. This gave no knowledge about their activity during the 24 hours; whether these insects remained in a given treatment for 24 hours after once entering or whether they may have moved to other locations. Measures that could indirectly indicate activity or movement are the oviposition and later

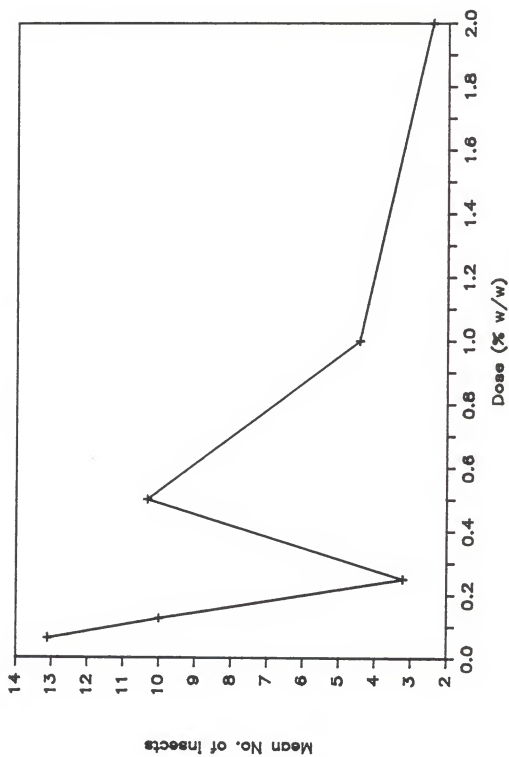


Figure V: Mean numbers of nonrespondent adult RW after 24 hour repellency test at different dose levels

development of insects in these samples. These samples were also used to determine whether treatments were likely to reduce development in treated wheat.

Mean numbers of adult insects emerged from samples at three dose levels (2.0, 1.0 and 0.5%) after 60 days of incubation are given in Appendices III and IV for leaf and flower powder treatments, respectively. The statistical comparison of treated and untreated samples for F_1 emergence (Table 6) indicated that numbers of insects emerged in treated samples were significantly lower than for untreated samples for both insect species. This indicates that insects were more likely to infest and multiply in untreated grain. During the free choice test it appeared that treatments repelled the insects and discouraged them from laying eggs. It appeared that white sagebrush has the potential to repel insects and to significantly limit the multiplication of RW and LGB in grain in storage.

Mean numbers of insects emerged were higher for flowers than for leaves, both in treated and untreated samples and for both species of insects (Table 6). Although there is the temptation to think of higher reproductivity in grain treated with flower powder as being a stimulatory effect, this is probably not the case. Leaves and flowers were tested and incubated at different times during the

Table 6: Mean number of adult RW and LGB emerged after 60 days of incubation in samples treated with powders of leaves and flowers of white sagebrush

Samples	Mean No. of adults emerged*			
	Rice Weevil		Lesser Grain Borer	
	Leaves	Flowers	Leaves	Flowers
Treated	3.22 a	8.33 a	3.33 a	6.89 a
Untreated	79.56 b	131.33 b	54.00 b	109.78 b

* Each value is a mean of 9 observations

Means within columns having same letter are not significantly different at $\alpha = 0.05$

study, and this difference in numbers of adults emerged may have been due to the difference in population of insects used for tests. Insect populations used for leaf powder testing could have been of lower fecundity than those used for flower powder testing. This aspect should be investigated further.

(3) Persistence of repellent activity

A third aspect of this study was to observe the persistence of repellent action of white sagebrush powder over a storage period of 4 months. Only flower powder was used, because it proved to be a significantly better repellent than the two other parts for RW. Three doses (1.0, 0.25 and 0.125%) based on previous results, were used to measure the persistence effect. Percent repellency was determined initially and after each one month interval (Appendix V). Mean percent repellency of RW tended to decrease rapidly for the lower two dose levels (0.25 and 0.125%) , while the 1% dose gave good repellency values. The 1% dose of flower powder was fairly persistent in repellent action over a period of four months storage decreasing to about 75% of the original value (Fig. VI). At 0.25 and 0.125% repellencies dropped to 40 and 27% of initial values, respectively. For LGB the repellency at a dose of 1.0% dropped to 65% of the initial value. At the

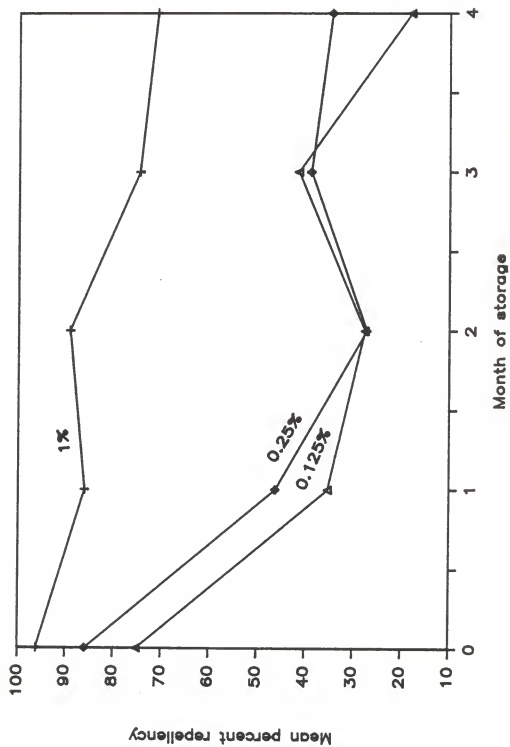


Figure VI: Persistence of mean percent repellency of powder of flowers of white sagebrush at three dose levels against RW

0.25% treatment level, repellency of LGB appeared to be unchanged over time and at the 0.125% dosage, repellency was greater than the initial value (Fig. VII).

Statistically it was found that dose (Table 7) and different time periods (Table 8) were significantly different in their percent repellency values. However, there was no significant interaction found between dose levels and different time intervals against RW ($F = 1.78$; $d.f = 8$; $P > 0.1314$). There was an obvious interaction for the LGB ($F = 3.41$; $d.f = 8$; $P > 0.0094$). A comparison of mean percent repellency after each month during storage up to 4 months indicated that initial mean percent repellency values for RW were significantly higher than the succeeding months (Table 8). Repellency dropped sharply after the first month. After 1, 2 and 3 months there was no statistical difference in mean percent repellency. The lowest value was obtained after four months. For LGB repellency values fluctuated with virtually no difference between the initial and different time intervals. Mean percent repellency values for 0, 1, and 2 months were not statistically different (Table 8). The Value for month 3 was significantly different from the initial and month 2, however, it was statistically the same as for months 1 and 4.

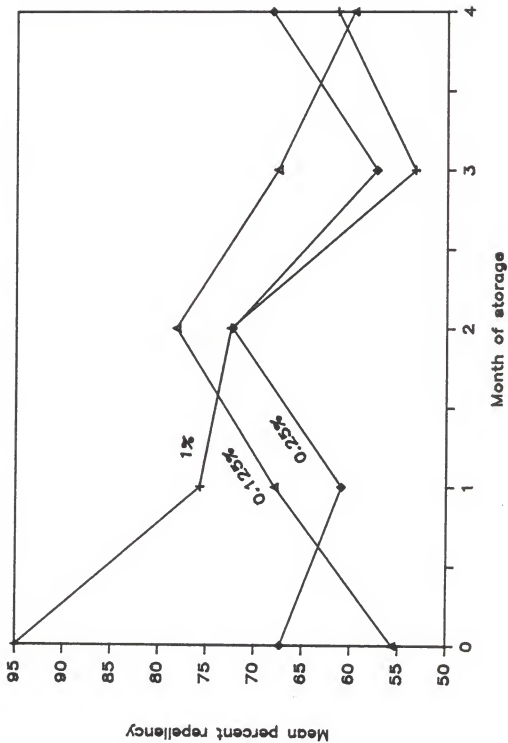


Figure VII: Persistence of mean percent repellency of powder of flowers of white sagebrush at three dose levels against LGB

Table 7: Statistical comparison of mean percent repellency of powder of flowers of white sagebrush against RW and LGB at three dose levels.

Dose (% w/w)	Mean percent repellency*	
	Rice weevil	Lesser grain borer
1.0	83.38 a	71.59 a
0.25	46.58 b	65.83 a
0.125	39.50 b	65.25 a

* Each value is a mean of 15 observations

Means within columns having same letter are not significantly different at $\alpha = 0.05$

Table 8: Statistical comparison of mean percent repellency of powder of flowers of white sagebrush against RW and LGB after different storage periods.

Storage time (months)	Mean percent repellency*	
	Rice weevil	Lesser grain borer
0	85.71 a	72.63 ab
1	55.86 b	68.19 abc
2	48.00 bc	74.37 a
3	51.67 bc	59.44 c
4	41.18 c	63.15 bc

* Each value is a mean of 9 observations

Means within columns having same letter are not significantly different at $\alpha = 0.05$

Comparison of interaction between dose levels and different storage times against LGB is given in Table 9. It was concluded from the persistence study that although initial mean percent repellency of flower powder was very high, it decreased rapidly during storage except at the 1% dose level for RW, which was fairly persistent over four month storage.

Jotwani and Sircar (1965) reported that neem seed powder mixed with wheat at the rate of 1 percent protected treated wheat against RW and LGB for about 9 and 10 months, respectively. Flower powder of white sagebrush used at the rate of 1 percent in studies reported here showed fairly persistent repellent action against RW for four months decreasing to about 75% of the original value. However, repellency for LGB dropped to 65% of the initial value. Ketkar (1976) observed reduction in population of RW and LGB in paddy grain mixed with powder of neem seed at the rates of 0.5, 0.75 and 1.0 percent. Similarly neem leaf/seed powder when mixed with shelled maize resulted in reduced F_1 progeny of RW and LGB (Pereira and Wohlgemuth, 1982). Results reported here for flower/leaf powder of white sagebrush mixed with wheat at the rates of 2, 1 and 0.5 percent also significantly reduced the F_1 emergence of RW and LGB, indicating that white sagebrush can be a potential protectant of stored-grain.

Table 2: Statistical comparison of mean percent repellency of powder of flowers of white sagebrush against LGB at three dose levels after different storage periods.

Storage time (months)	Mean percent repellency* at dose level of		
	1% w/w	0.25% w/w	0.125% w/w
0	94.99 a	67.30 bcd	55.59 de
1	75.69 bc	60.95 bcd	67.94 bcde
2	72.52 bcd	72.38 bcd	78.22 ab
3	53.33 e	57.32 de	67.66 bcde
4	61.44 bcde	68.29 bcde	59.74 cde

* Each value is a mean of 3 observations

Means having same letter are not significantly different at $\alpha = 0.05$

It has been suggested by different authors (Qadri, 1973; Golob and Webley, 1980; and Su et. al, 1982) that most of the repellents from indigenous plant materials act in the vapor phase. Examples of such plants are: Curcuma Spp., Acorus Spp., citronella, pines and arrow root. The source of the repellent action of powders of different plant parts of white sagebrush is not known but may be due to some volatile chemicals acting in the vapor phase. More detailed studies investigating the source of repellency of these plant materials are required to more completely understand their action. Knutson (1982) related antifeedant value of this plant to the presence of pubescence on leaves. He suggested that dense pubescence present on leaves prevented grasshoppers from feeding on them. Studies conducted here were not designed to determine a possible relationship between the presence of pubescence and repellent action. Further studies appear warranted based on data presented here.

CONCLUSIONS

This study was undertaken for preliminary evaluation of the repellent action of Artemisia ludoviciana plant powders against two species of stored-grain insects. The following conclusions were drawn from this study:

- 1) White sagebrush (Artemisia ludoviciana) showed promising repellent properties against RW and LGB, when used in a powdered form.
- 2) All three growth stages of plant showed some repellency, but flowers were most promising.
- 3) Numbers of insects found outside the cups (nonrespondents) during free choice tests did not affect the repellency values determined by the technique used.
- 4) Incubation studies indicated that numbers of F_1 adults emerged from treated samples were significantly lower than from untreated grain.
- 5) At 1%, flower powder repellency of RW was fairly persistent over four months storage. Although flower powder at 0.25 and 0.125% dose levels showed promising repellent action initially, its effectiveness decreased abruptly after one month storage.

- 6) More detailed studies are required to investigate the actual factor(s) responsible for repellent action and their chemical and/or physical properties.

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APPENDICES

Appendix I

Mean percent repellency of different parts of white sagebrush at six dose levels against RW

Dose (% w/w)	Mean % Repellency*		
	Whole plant	Leaves	Flowers
2.0	99.44	99.44	97.73
1.0	97.70	93.25	94.56
0.5	94.86	86.90	93.10
0.25	91.66	84.73	96.07
0.125	74.48	84.29	95.62
0.0625	62.11	62.01	75.47

* Each value is a mean of three replicates

Appendix II

Mean percent repellency of different parts of white sagebrush at six dose levels against LGB

Dose (% w/w)	Mean % Repellency *		
	Whole plant	Leaves	Flowers
2.0	93.86	92.12	89.13
1.0	79.20	61.92	91.96
0.5	65.06	62.36	70.64
0.25	68.70	41.95	72.64
0.125	61.65	39.86	66.88
0.0625	61.70	24.24	52.26

* Each value is a mean of three replicates

Appendix III

Mean number of adult RW and LGB emerged after 60 days of incubation in the samples treated with powder of leaves of white sagebrush at three dose levels

Insect	Dose (% w/w)	No.* of adults emerged in	
		Treated samples	Untreated samples
Rice Weevil	2.0	0.33	87.33
	1.0	5.00	73.33
	0.5	4.33	78.00
Lesser Grain Borer	2.0	2.00	59.67
	1.0	5.00	35.67
	0.5	3.00	66.67

* Each value is a mean of three replicates

Appendix IV

Mean number of adult RW and LGB emerged after 60 days of incubation in the samples treated with powder of flowers of white sagebrush at three dose levels

Insect	Dose (% w/w)	No.* of adults emerged in	
		Treated samples	Untreated samples
Rice Weevil	2.0	10.33	120.00
	1.0	7.00	128.33
	0.5	7.67	145.67
Lesser Grain Borer	2.0	11.67	130.67
	1.0	1.00	84.67
	0.5	8.00	114.00

* Each value is a mean of three replicates

Appendix V:

Repellency values of flower powder of white sagebrush against RW and LGB at three dose levels after different storage periods.

Insect	Dose (% w/w)	Percent repellency											
		Initial			1 month						2 month		
		R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3
RW	1	98.31	96.66	93.33	91.66	91.66	91.66	81.03	96.66	89.83			
	0.25	86.55	88.23	83.05	32.77	64.40	41.66	47.82	28.81	5.08			
	0.125	65.81	77.96	81.51	5.17	40.67	60.00	29.91	40.86	12.06			
LGB	1	91.66	95.00	98.30	62.71	84.87	79.48	64.70	63.02	89.83			
	0.25	66.10	79.13	56.66	59.32	57.14	66.38	71.66	79.66	65.81			
	0.125	49.15	68.06	49.57	50.84	73.33	79.66	78.33	86.32	70.00			

..... Continued on next page

Insect	Dose (% w/w)	Percent repellency					
		3 month			4 month		
		R1	R2	R3	R1	R2	R3
RW	1	67.52	75.00	81.35	78.33	57.98	76.66
	0.25	53.33	19.33	44.06	37.28	51.26	15.00
	0.125	31.03	43.85	49.57	9.56	9.56	35.00
LGB	1	61.06	24.36	74.57	59.32	46.66	78.33
	0.25	61.40	55.00	55.55	81.96	73.3	49.57
	0.125	63.33	69.49	70.17	45.00	72.88	61.34

R1- R3 = Replicates

Repellent properties of white sagebrush (Artemisia ludoviciana Nutt. (Asteraceae)) against rice weevil (Sitophilus oryzae (L.)) and lesser grain borer (Rhyzopertha dominica (F.))

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ABSTRACT

Kansas white sagebrush (Artemisia ludoviciana Nutt.) was evaluated as a repellent against two species of stored-grain insects: rice weevil (Sitophilus oryzae (L.)) and lesser grain borer (Rhyzopertha dominica (F.)). Plants were collected from two different locations near Manhattan, Kansas in three growth stages: early growth (less than 15 cm in height), fully grown plant with leaves and matured plant with flowers. Dried plant material was pulverized on a Wiley mill using a 1 mm screen to get a unifrom powder. Rice weevil and lesser grain borer were reared and tested on hard red winter wheat at $27 \pm 1^{\circ}$ C temperature and $60 \pm 5\%$ R.H.

Repellent properties of powders of whole early growth stage, leaves of full grown plants and flowers were evaluated using a modified choice chamber. Repellency over 24 hours was determined at six dose levels: 2, 1, 0.5, 0.25, 0.125 and 0.0625% (w/w). Numbers of adult insects emerged from samples exposed to the insects in a choice test for 24 hours were also determined. Persistence of repellent action of powder of flowers at 1.0, 0.25 and 0.125% (w/w) over a 4-month storage period was determined.

Powders of all plant parts showed repellent action but that of flowers was significantly better than that of whole

plant and leaves against both insect species. Immediately after treatment, rice weevil repellency was greater than 90% at 0.125%. Lesser grain borer repellency was not as great but tended to increase with dosage. F_1 adults emerged after 60 days from wheat treated with powders of leaves and flowers at three dose levels were significantly fewer than from untreated samples.

At 1%, flower repellency values for rice weevil decreased from 96.10 to 70.99% during four months storage, while it decreased sharply after 1 month at 0.25 and 0.125%. Repellency values for lesser grain borer were lower and not significantly different over time.